

[Abstract]

The present invention concerns methods for the ex vivo formation of mammalian bone and subsequent uses of the bone. A critical and distinguishing feature of the present invention are defined tissue culture conditions and factors resulting in the formation of bone cell spheroids. The invention also provides for methods of implanting into subjects the ex vivo formed bone. Also described are methods for genetically altering the bone cell spheroids to affect bone formation, identification of candidate modulators of bone formation, and identification of genes involved in bone formation.

[Claims]

1. A method for producing bone ex vivo, comprising the steps of : a) obtaining an osteogenic cell or bone precursor cell ; b) culturing said cell under serum free conditions in the presence of one or more osteogenic growth factors ; and c) maintaining the cell cultures at cell densities that allow the formation of a bone cell spheroid, whereby bone is formed by cells within said bone cell spheroid.
2. The method of claim 1, wherein the osteogenic cell or bone precursor cell is of human origin.
3. The method of claim 1, wherein the osteogenic cell or bone precursor cell is of bovine origin.
4. The method of claim 1, wherein the osteogenic cell or bone precursor cell is of equine origin.
5. The method of claim 1, wherein the osteogenic cell or bone precursor cell is of canine origin.
6. The method of claim 1, wherein the osteogenic cell or bone precursor cell is of feline origin.
7. The method of claim 1, wherein the osteogenic cell or bone precursor cell is of murine origin.
8. The method of claim 1, wherein the osteogenic cell or bone precursor cell is of rat origin.
9. The method of claim 1, wherein the osteogenic cell or bone precursor cell is of chick origin.
10. The method of claim 1, wherein the growth factor is TGF- β 1, TGF- β 2, TGF- β 1, 2, VEGF, insulin-like growth factor I or II, BMP2, BMP4, or BMP7.
11. The method of claim 1, wherein the growth factor is parathyroid hormone, calcitonin, interleukin-6, or interleukin-11.
12. The method of claim 1, further comprising purifying the osteogenic cell or bone precursor cell by physico-chemical separation techniques.
13. The method of claim 12, wherein the physico-chemical separation technique is equilibrium density separation.
14. The method of claim 1, further comprising purifying the osteogenic cell or bone precursor cell by immuno-affinity isolation.
15. The method of claim 14, wherein the immuno-affinity isolation utilizes immune adhesion, immuno-column chromatography, or fluorescence-activated cell sorting.
16. The method of claim 14, wherein the immuno-affinity isolation utilizes antibodies to osteocalcin, osteonectin, or alkaline phosphatase, or combinations thereof.

17. The method of claim 1, wherein said cell-densities at the initiation of the culture are from about 1.0×10^3 to about 1×10^6 cells per cm^2 .
18. The method of claim 1, further comprising implanting the cells *in vivo*.
19. A method of providing bone tissue to a mammal, comprising obtaining a bone cell spheroid and implanting the bone cell spheroid into said mammal.
20. The method of claim 19, wherein the bone cell spheroid is implanted in one or more of alginate gels, collagen gels, or fibrin gels.
21. The method of claim 19, wherein the bone cell spheroid is implanted in one or more of polylactic acid, polyglycolic acid or PGLA.
22. The method of claim 19, wherein the bone cell spheroid is implanted in or in conjunction with hydroxyapatitic, other apatitic compounds, devitalized animal bone, devitalized human bone, or porous ceramic structures.
23. The method of claim 19, wherein the implantation is made in conjunction with orthopedic surgery and/or orthopedic devices, such as hip implants, knee implants, or spinal fusions.
24. The method of claim 19, wherein the implantation is made in conjunction with oral surgery and/or dental implants.
25. The method of claim 19, wherein the implantation is made in conjunction with plastic surgery.
26. The method of claim 19, wherein the implantation is in conjunction with periodontal repairs.
27. The method of claim 19, wherein the implantation is into bone-forming tissue.
28. The method of claim 19, wherein the implantation is into a wound.
29. The method of claim 19, wherein the mammal has a bone disease such as osteoporosis, Vitamin D deficiency, Osteotitis deformans, Von Recklinghausen's Disease.
30. A method for producing bone *ex vivo*, comprising the steps of : a) obtaining an osteogenic cell or bone precursor cell ; b) culturing said cell under serum free conditions in the presence of one or more osteogenic growth factors ; c) maintaining the cell cultures at cell-densities that allow the formation of a bone cell spheroid, whereby bone is formed by cells within said bone cell spheroid ; and, d) removing the cellular elements from the formed bone cell spheroid and using resulting bone *in vivo*.
31. A method for producing bone *ex vivo*, comprising the steps of : a) obtaining an osteogenic cell or bone precursor cell ; b) culturing said cell under serum free conditions in the presence of one or more osteogenic growth factors ; c) contacting said cell with a recombinant vector that directs the expression of a protein that modifies bone cell spheroid formation ; and d) initiating the cell cultures at cell-densities that allow the formation of a bone cell spheroid whereby bone is formed by cells within said bone cell spheroid.
32. The method of claim 31, wherein said protein that enhances bone cell spheroid formation is TGF- β 1, TGF- β 2, TGF- β 1, 2, PTH, calcitonin, interleukin-6 or interleukin-11, BMP2, BMP4, BMP7, collagen, osteonectin, osteopontin, bone sialoprotein, osteocalcin, insulin- like growth factors I or II, VEGF, integrin α chains, integrin β chains, selectins, fibronectin, thrombospondin or cadherin.
33. A method for using bone for bone repair in a subject, comprising the steps of : a) obtaining an osteogenic cell or bone precursor cell ; b) culturing said cell under serum free conditions in the presence of one or more osteogenic growth factors ; and, c) contacting said cell with a recombinant vector that expresses a protein that enhances bone cell spheroid formation ; d) maintaining the cell cultures at cell-densities that allow the formation of a bone cell spheroid, whereby bone is formed by cells within

said bone cell spheroid ; e) removing the cellular elements from the ex vivo formed bone ; and f) using said bone to effect repair.

34. A method for identifying a gene involved in bone formation, bone repair and/or bone disease, comprising the steps of : a) obtaining an osteogenic cell or bone precursor cell ; b) culturing said cell under serum free conditions in the presence of one or more growth factors of the TGF- β gene superfamily ; c) maintaining the cell cultures at cell-densities that allow the formation of a bone cell spheroid ; and d) identifying a gene that is over or under expressed during the formation of a bone cell spheroid and not so expressed in the untreated osteogenic or bone precursor cell.

35. A method for identifying a modulator of bone formation, bone repair and/or bone disease, comprising the steps of : a) obtaining an osteogenic cell or bone precursor cell ; b) culturing said cell under serum free conditions in the presence of a candidate modulator in the absence of one or more osteogenic growth factors ; c) measuring bone cell spheroid formation ; and d) comparing the formation of bone cell spheroid with that observed in the absence of the modulator.

36. The method of claim 35, further comprising a step of culturing an osteogenic cell or bone precursor cell in the presence of one or more osteogenic growth factors.

37. A method for producing a modulator of bone formation, bone repair and/or bone disease comprising the steps of : a) obtaining an osteogenic cell or bone precursor cell ; b) culturing said cell under serum free conditions in the presence of a candidate modulator in the presence of one or more osteogenic growth factors ; c) measuring bone cell spheroid formation ; d) comparing the formation of bone cell spheroid with that observed in the absence of the modulator ; and e) producing a modulator so identified.

38. A bone cell spheroid made by the process of : a) obtaining an osteogenic cell or bone precursor cell ; b) culturing said cell under serum free conditions in the presence of one or more osteogenic growth factors ; and c) maintaining the cell cultures at cell densities that allow the formation of a bone cell spheroid, whereby bone is formed by cells within said bone cell spheroid.

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